

# Precision medicine approaches for the management of Ewing sarcoma: current perspectives

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**Abstract:** Advancements in molecular and genetic techniques have significantly furthered our biological understanding of Ewing sarcoma (ES). ES is typified by a driving TET–ETS fusion with an otherwise relatively quiet genome. Detection of one of several characteristic fusions, most commonly *EWSR1–FLII*, is the gold standard for diagnosis. We discuss the current role of precision medicine in the diagnosis, treatment, and monitoring of ES. Continued efforts toward molecularly guided approaches are actively being pursued in ES to better refine prognosis, identify germline markers of disease susceptibility, influence therapeutic selection, effectively monitor disease activity in real time, and identify genetic and immunotherapeutic targets for therapeutic development.

**Keywords:** Ewing sarcoma, next generation sequencing, genomics, liquid biopsy, targeted therapy

## Introduction

Sarcomas are a heterogeneous group of mesenchymal neoplasms that account for 1% of all adult malignancies, with >50 histologic subtypes identified and a reported incidence of 13,040 cases in 2018.<sup>1</sup> Ewing sarcoma (ES) as well as the more encompassing classification Ewing sarcoma family of tumors (ESFT) represents a significant percentage of bone sarcomas mainly effecting the pediatric and adolescent population with a mild predominance in the male gender. Despite an overall improvement in sarcoma-related deaths over the last two decades, patients presenting with more advanced ES continue to have a poor prognosis.<sup>2</sup>

The genetic profile of ESFT is dominated by the driving reciprocal translocation between *EWSR1* and *ETS* family transcription factors. Continuing research has provided insight into the prognostic value of certain genetic alterations reported in ES, as well as the potential future targets to further individual treatment regimens.<sup>3</sup> In this report, we review the molecular landscape of ES with a focus on translational aspects. We discuss the current role of precision medicine in the diagnosis, prognosis, and management of ESFT.

## Genomic landscape

Several large next-generation sequencing (NGS) studies have helped to define the genomic landscape of ES.<sup>4–6</sup> ES is a fusion-associated malignancy with recurrent translocations between *EWSR1* and members of the *ETS* family of transcription factors, most commonly *FLII*.<sup>7</sup> Similar to other fusion-driven malignancies,<sup>8</sup> ESFT is

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characterized by a low somatic mutational burden suggesting a primacy of the *EWSR1*–ETS fusion as a driver. Recurrent oncogenic mutations have been noted, however, in several tumor suppressors including *STAG2* (15%–22%), *TP53* (6%–7%), and *CDKN2A* (12%–28%).<sup>4–6</sup> Of note, ESFT displays a stark paucity of mutations in genes involved in kinase signaling pathways. Epigenomic and transcriptomic mapping have provided genome-wide overviews of the ES cellular program.<sup>9</sup> Indirectly targeting the fusion via associated epigenomic or transcriptomic changes has been challenging, but remains an area of active research.<sup>10</sup>

## Germline genetics

Germline genetics may play a role in the development of ES and can have important implications for the patient as well as the family members. A large case–control study of 1,162 patients with a variety of sarcoma subtypes found a high burden of likely pathogenic germline alterations in sarcoma patients relative to a control population with variants in *TP53*, *ATM*, *ATR*, *BRCA2*, and *ERCC2* being commonly represented.<sup>11</sup> Focusing on the ES population, a germline sequencing study utilizing whole-genome or whole-exome sequencing in 175 patients with ES identified pathogenic or likely pathogenic mutations in 13.1% of patients, with significant enrichment in genes involved in hereditary breast cancer and DNA repair pathways.<sup>12</sup> A separate germline analysis that included 46 pediatric and adolescent patients with ES also found 5 patients with damaging alterations in one of *TP53*, *PMS2*, or *RET*.<sup>13</sup> Germline alterations in more common cancers such as ovarian, breast, and colon have led to personalized medicine strategies unique to the individual's cancer biology. Given the overlap in genetic pathways seen in ES, these germline findings might benefit ES survivors and their families though the utilization of existing screening and surgical risk reduction strategies for genes such as *BRCA1/2*, *APC*, and *TP53*. Furthermore, germline variants may suggest clinical use of novel targeted therapies, ideally in the setting of a clinical trial. As examples, carriers of *BRCA1* or *BRCA2* deleterious germline mutations may benefit from PARP inhibitors, even outside of breast or ovarian cancer.<sup>14</sup>

## Diagnosis

Morphologically, ES family tumors can closely resemble other small round blue cell tumors. While histology and immunohistochemistry can be suggestive, it is now considered standard of care to confirm the diagnosis by detection of one of the typical ESFT-associated translocations.

More than 85% of Ewing's sarcomas are defined by the t(11;22)(q24;q12) translocation that fuses the *EWSR1* gene on chromosome 22 with the *FLI1* gene on chromosome 11.<sup>15</sup> The remaining 10%–15% of cases are characterized by alternate translocations resulting in the *EWSR1* gene instead being fused with other ETS transcription factors including *ERG*, *ETV1*, *ETV4*, or *FEV* or rarely by *EWSR1* being replaced by another member of the TET family of transcription factors, *FUS*<sup>16</sup> (Table 1).

Clinical molecular testing for ESFT translocations is most commonly performed using fluorescence in-situ hybridization (FISH) or reverse transcription-PCR (RT-PCR). Due to the limitations of these technologies, most molecular pathology laboratories do not test for all of the less common TET–ETS fusion pairings. Therefore, a negative FISH or RT-PCR does not rule out the diagnosis of ESFT and more extensive molecular evaluation (eg, NGS) may be necessary to solidify the diagnosis. An increasing number of alternate fusion drivers have been characterized in “Ewing-like” tumors, including CIC–DUX4 or CIC–FOXO4 and BCOR–CCNB3.<sup>17–20</sup> Though sometimes designated as “Ewing family” or “Ewing-like” tumors, gene expression profiling of these non-TET–ETS fusion sarcomas indicate they are molecularly distinct from ESFT and should probably be considered a different entity.<sup>4,19</sup>

## Treatment

Primary treatment for localized ES includes multi-agent neo-adjuvant chemotherapy, most commonly vincristine, doxorubicin, and cyclophosphamide alternating with ifosfamide and etoposide, given every 2 weeks. This is then followed by local therapy (surgery and/or radiation) followed by adjuvant chemotherapy. Following this rigorous regimen, patients with localized disease have a survival rate of ~70%.<sup>21,22</sup> Unfortunately, patients who develop recurrence in addition to the 20%–30% of patients who present with metastatic disease at diagnosis portend a dismal prognosis with a 5-year overall survival of <20%.<sup>23,24</sup>

**Table 1** Current TET–ETS fusion pairings identified in Ewing sarcoma

Translocation	Fusion	Frequency in ESFT
t(11;22)(q24;q12)	<i>EWSR1</i> – <i>FLI1</i>	~85%–90% of cases
t(21;22)(q22;q12)	<i>EWSR1</i> – <i>ERG</i>	~10% of cases
t(7;22)(p22;q12)	<i>EWSR1</i> – <i>ETV1</i>	Rare
t(17;22)(q12;q12)	<i>EWSR1</i> – <i>ETV4</i>	Rare
t(2;22)(q35;q12)	<i>EWSR1</i> – <i>FEV</i>	Rare
t(16;21)(p11;q22)	<i>FUS</i> – <i>ERG</i>	Rare
t(2;16)(q35;p11)	<i>FUS</i> – <i>FEV</i>	Rare

**Abbreviation:** ESFT, Ewing sarcoma family of tumors.

## Molecularly targeted therapy

While there have been clear advancements in the use of genetic profiling for the diagnosis of ES, the translation of genomic knowledge into therapeutic advances has been more challenging. Unlike kinase fusions, the transcription factor fusions such as *EWSR1-FLI1* have not been amenable to direct inhibition to date. Furthermore, some evidence suggests that EWS-FLI1-directed oncogenesis in ES involves the fusion oncoprotein downregulating rather than upregulating genetic pathways, making targeting downstream effects of the fusion a challenge as well.<sup>25</sup> A number of targeted therapy efforts are ongoing in attempts to overcome these challenges (Table 2).

## Insulin-like growth factor one receptor (IGF-1R) inhibitors

The insulin and IGF-1R pathway has a role in normal cellular development and has been associated with the development of malignancies such as prostate cancer,<sup>26</sup> breast cancer,<sup>27,28</sup> and colon cancer.<sup>29</sup> Since the IGF-1R pathway is deregulated by the *EWSR1-FLI1* translocation, it has come under the spotlight as a potential target for therapy. Based on promising preclinical evidence, several Phase I studies of this drug class have been completed with encouraging results and good tolerability.<sup>30–32</sup> In a 2016 review of IGF-1R studies in ES, 311 ES patients were treated with IGF-1R inhibitors as monotherapy on Phase I or Phase II trial. Of these, 2 (0.6%) had a complete response, 31 (9.9%) had a partial response, and 66 (21%) had stable disease.<sup>33–35</sup> Although preclinical data and an earlier Phase II trial suggested combination therapy may help overcome resistance,<sup>36</sup> a Phase II trial utilizing an IGF-1R inhibitor with an mTOR inhibitor unfortunately did not show any responses in the 43 eligible patients, including 11 ES patients.<sup>34,37</sup>

Given the modest disease activity of IGF-1R inhibitors as monotherapy, current efforts focus on whether there is a role for the addition of this targeted therapy to standard cytotoxic chemotherapy. A Phase III trial assessing the IGF-1R monoclonal antibody ganitumab in combination with chemotherapy in first-line treatment for metastatic ESFT is ongoing (NCT02306161).

## PARP inhibition

The PARP pathway is essential for genomic stability via detection of single-strand breaks and signaling of enzymatic DNA repair. A large systematic drug screen utilizing hundreds of cell lines identified ES as being exquisitely sensitive to the PARP inhibitor olaparib.<sup>38</sup> Mouse xenograft studies additionally support PARP inhibition, particularly in combination with irinotecan and temozolomide as a viable therapeutic strategy for this disease.<sup>39</sup> In a Phase II trial, 12 patients with ES who previously progressed on standard chemotherapy were treated with olaparib monotherapy at a dose of 400 mg by mouth twice daily. Although the drug was well tolerated, no complete or partial responses were seen in any of the patients. Stable disease was seen in 4 of the 12 patients (range 10.9–18.4 weeks), and median progression-free survival was 5.7 weeks.<sup>40</sup> An ongoing trial is assessing combination therapy with olaparib with temozolomide and irinotecan in recurrent/refractory ES (NCT01858168).

## LSD1 inhibition

LSD1, as a member of the NuRD co-repressor complex, is recruited by EWS-FLI1 to exert transcriptional regulation. In both in vitro and in vivo disease models, inhibition of LSD1 leads to a reversal of the EWS-FLI1 transcriptional profile and shows significant single-agent activity.<sup>41,42</sup> Reversible,

**Table 2** Current clinical trials for advanced Ewing sarcoma

NCT no.	Patient population	Phase	Treatment	Mechanism of action
NCT02306161	Newly diagnosed, metastatic disease	III	Standard chemotherapy ± ganitumab	IGF-1R mAb
NCT03495921	Recurrence after one prior therapy	III	Temozolomide and irinotecan ± vigil	bi-shRNA(furin) and GM-CSF augmented autologous tumor cell immunotherapy
NCT01858168	Recurrence after >1 prior therapy	I	Olaparib and temozolomide ± irinotecan	PARP inhibitor
NCT02736565	Relapsed or refractory disease	I	pbi-shRNA™ EWS/FLI1 Type I LPX	Functional plasmid DNA construct that targets EWS/FLI1 mRNA
NCT03514407	Relapsed or refractory disease	Ib	INCB059872	LSD1 inhibitor
NCT03600649	Relapsed or refractory disease	I	SP-2577 (seclidemstat)	LSD1 inhibitor
NCT02657005	Relapsed or refractory disease	I	TK216	ETS-family transcription inhibitor

**Abbreviations:** GM-CSF, granulocyte-macrophage colony-stimulating factor; IGF-1R, insulin-like growth factor one receptor; mAb, monoclonal antibody.

as opposed to irreversible LSD1 inhibition, in particular, seems promising based on xenograft models.<sup>42</sup> As a result of these preclinical efforts, several LSD1 inhibitors have recently begun testing in ES in early phase monotherapy trials (Table 2).

## Immunotherapy

Immunotherapy breakthroughs in a number of adult cancer types have sparked the interest in investigating this approach in ES as well. Unfortunately, early experience with checkpoint inhibitor therapy in ES has not shown significant efficacy to date. Most notably, 0 of 13 ES patients treated with pembrolizumab on the SARC028 trial experienced an objective response.<sup>43</sup> The limited success with the use of immune checkpoint inhibitors in ES has been attributed to low mutational burden and absence of PD-L1 expression in the tumor.<sup>44</sup>

Utilization of cellular and vaccine immunotherapy strategies has become a topic of interest in ES.<sup>45</sup> Recently, a pilot study utilizing an autologous vaccination strategy has shown promising response data<sup>46</sup> and has led to an ongoing randomized trial (NCT03495921) with other approaches continuing to be developed. Anecdotal clinical efficacy has been reported with transgenic T-cell infusion.<sup>47</sup> Chimeric antigen receptor T cells targeting IGF1R or ROR1 are also being investigated for their use in ES. Thus far, there has been success in ES mouse models leading to a prolonged overall survival, but results have been limited to preclinical studies.<sup>48</sup>

## Prognosis and disease monitoring

### Genetic prognostic markers

Prognostic biomarkers are useful for identifying patients who may not respond well to conventional therapy. As discussed above, the genomic landscape of ESFT is typified by a low somatic mutational burden and few recurrently mutated genes. Of the three most highly recurrent mutations in *STAG2*, *CDKN2A*, and *TP53*, there are conflicting data at present on the prognostic utility of these findings. Initial landscape genomics studies suggested that *STAG2* mutation, particularly the combination of *STAG2* and *TP53* mutations, is a poor prognostic indicator;<sup>4,12</sup> however, this finding was not confirmed in a follow-up study using an independent cohort.<sup>49</sup> Similarly, there are conflicting studies regarding whether *CDKN2A* deletion is associated with poor prognosis.<sup>50,51</sup> Given the conflicting data regarding prognostic implications of the most common genetic alterations, further research is necessary prior to incorporation in standard clinical practice.

## Liquid biopsy

Of growing interest in precision oncology is the use of “liquid biopsy” techniques to monitor disease response and for early detection of relapse. Given that presence of TET–ETS fusion is a requisite for ESFT, detection and monitoring of the fusion transcript or from circulating tumor DNA (ctDNA) in the peripheral blood is a logical approach.

Several efforts utilizing RT-PCR methodology have shown the ability to detect fusion transcript in the peripheral blood or bone marrow of patients with both localized and metastatic disease.<sup>52–56</sup> The prognostic value and utility of this finding, however, was not conclusive between studies, and this technique has not been widely adopted.

More recently, technological advances in detection of plasma-derived cell-free tumor DNA by capture-based NGS or digital droplet PCR (ddPCR) have reignited the interest in plasma-based detection of the ES fusion as a disease monitoring tool with intriguing, although early, results. In a 94-patient ES cohort using an NGS hybrid capture assay, ctDNA was identified in 53% of newly diagnosed patients with localized disease.<sup>57</sup> The presence of detectable ctDNA by this assay was associated with worse 3-year event-free survival (48.6% vs 82.1%,  $P=0.006$ ) and overall survival (79.8% vs 92.6%,  $P=0.01$ ).

Several groups have now shown ddPCR to be a highly sensitive and dynamic plasma-based detection tool for the EWS fusion.<sup>58–60</sup> Though modest in patient numbers, these initial efforts have consistently shown nearly 100% sensitivity for detection in patients with measurable disease. Furthermore, the quantity of ctDNA was noted to be dynamic, correlating well with disease burden. Interestingly, ctDNA detection by this method can precede radiographic disease progression, suggesting a role for minimal residual disease monitoring.

While these initial efforts are intriguing, further investigation is needed to clarify how these technologies might be incorporated into clinical practice. At present, ctDNA detection in ES patients is limited to the research setting, but it is increasingly being included as a translational component in collaborative group trials.

## Conclusion

Advancements in molecular and genetic techniques have significantly furthered our biological understanding of ES. ES is typified by a driving TET–ETS fusion with an otherwise relatively quiet genome. Detection of one of several characteristic fusions, most commonly *EWSR1–FLI1*, is the gold standard for diagnosis. Despite a lack of driving



kinase pathway mutations, functional genomics studies have helped to identify recurrently aberrant pathways as well as genetic and epigenetic vulnerabilities that have led to ongoing investigations with precision therapy approaches. Detection of ctDNA in the plasma of ES patients represents a potential novel disease monitoring tool. Germline genomics studies have recently identified a higher than anticipated burden of pathogenic syndromic mutations in ES patients, often with clinical implications. Although the driving mutation and the gene expression profile have been more clearly defined, there has been a limited role of precision medicine in ES when compared to other malignancies. Continued efforts toward precision medicine approaches are actively being pursued in ES to better refine prognosis, identify germline markers of disease susceptibility, influence therapeutic selection, effectively monitor disease activity in real time, and to identify genetic and immunotherapeutic targets for therapeutic development.

## Disclosure

ASB – a family member has provided expert testimony for GlaxoSmithKline. The other authors report no conflicts of interest in this work.

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